Combining Multiplexed Gel Capillary Electrophoresis With Liquid Chromatography for Offline Comprehensive Analysis of Complex Oligonucleotide Samples

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Offline two dimensional liquid chromatography (LC) x capillary gel electrophoresis (CGE) and LC x (24) multiplexed-CGE methodologies were developed for the separation of oligonucleotides of therapeutic size. Both ion-pairing reversed phase liquid chromatography (IP-RPLC) and ion exchange liquid chromatography (IEX-LC) were studied as methods for the first dimension and single and multiplexed capillary electrophoresis methods in entangled polymer solutions were used for the second dimension separations. Electrokinetic and pressure injection were evaluated for the analysis of the collected LC fractions. The comprehensive separation was optimized with standard mixtures of poly adenosine, thymidine, cytosine and uracil homodeoxyoligonucleotides up to 35 bases long. Highly orthogonal methodologies and overall peak capacities of 6435 and 6993 for IP-RPLC x CGE and IEX-LC x CGE, respectively, were obtained within a few hours analysis time.